Appendix A.

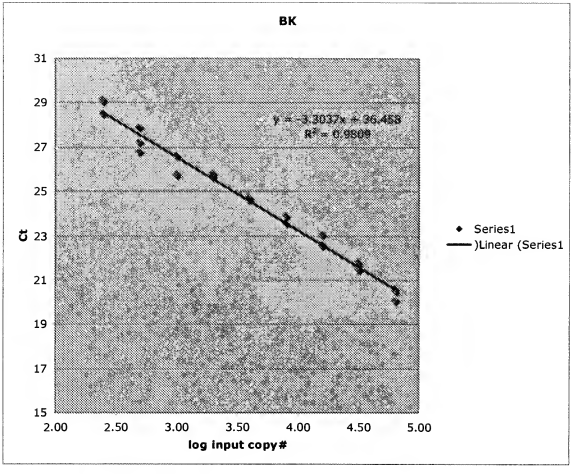
Table 1: Input copy numbers and measured Cts (Threshold Cycles) used for generation of the calibration plots for multiplex viral load measurements.
Triplicate sets of experiments are shown.

		Measured Threshold Cycles (Ct)			
input copy #	log(input)	BK	HHV7	CMV	HHV6b
62500.0	4.80	20.122	21.739	22.79	21.129
31250.0	4.49	21.84	23.43	24.207	22.691
15625.0	4.19	23.046	24.305	25.327	23.436
7812.5	3.89	23.92	25.651	26.653	24.667
3906.3	3.59	24.741	26.722	27.919	25.827
1953.1	3.29	25.755	24.673	28.261	26.648
976.6	2.99	26.644	27.753	29.852	28.515
488.3	2.69	27.916	29.627	30.799	28.781
244.1	2.39	29.106	28.581	30.019	29.74
62500.0	4.80	20.652	22.504	23.956	21.466
31250.0	4.49	21.463	23.522	24.6	23.041
15625.0	4.19	22.644	24.094	25.353	24.045
7812.5	3.89	23.575	24.784	26.888	25.282
3906.3	3.59	24.714	26.831	28.074	25.794
1953.1	3.29	25.657	26.639	28.507	26.716
976.6	2.99	25.735	27.287	28.871	26.938
488.3	2.69	26.834	28.394	29.252	27.778
244.1	2.39	28.53	30.343	31.69	29.644
62500.0	4.80	20.529	22.542	23.895	22.066
31250.0	4.49	21.694	23.527	24.742	23.098
15625.0	4.19	22.56	24.295	25.69	23.986
7812.5	3.89	23.587	25.492	26.783	25.202
3906.3	3.59	24.652	26.865	27.834	25.845
1953.1	3.29	25.842	27.407	28.629	27.057
976.6	2.99	25.81	27.675	29.971	27.804
488.3	2.69	27.271	28.901	30.418	28.28
244.1	2.39	29.139	30.524	31.746	29.537
	slope	-3.3036575	-3.0047762	-3.1694331	-3.2096654
	intercept	36.4577227	36.8704722	38.8922137	37.2695701

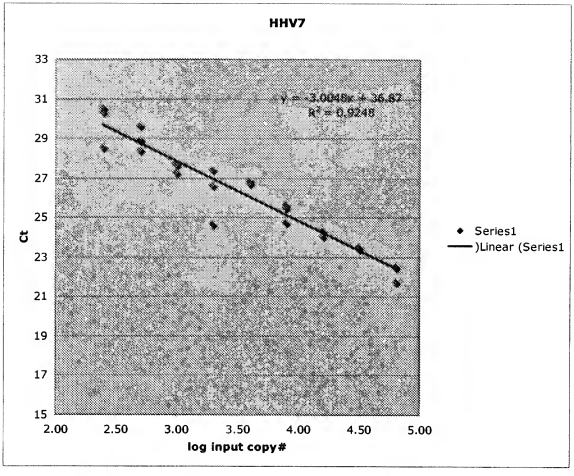
Figure 1:

Calibration plots for multiplex viral quantification:

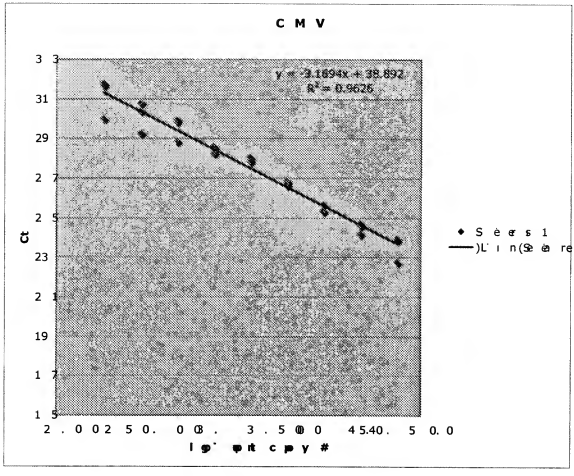
Calibration plot for BK virus:



Calibration plot for HHV7 virus



Calibration plot for CMV virus



Calibration plot for HHV6 virus

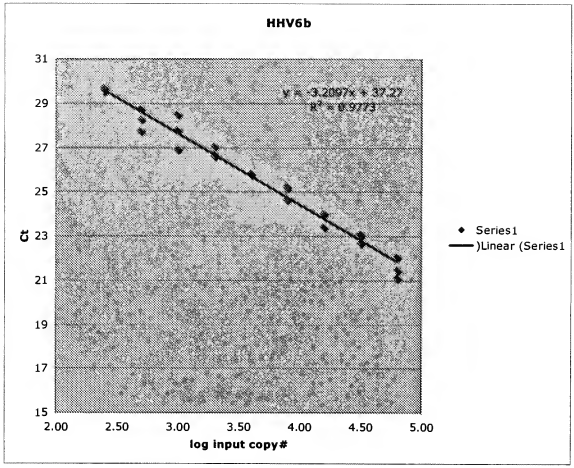
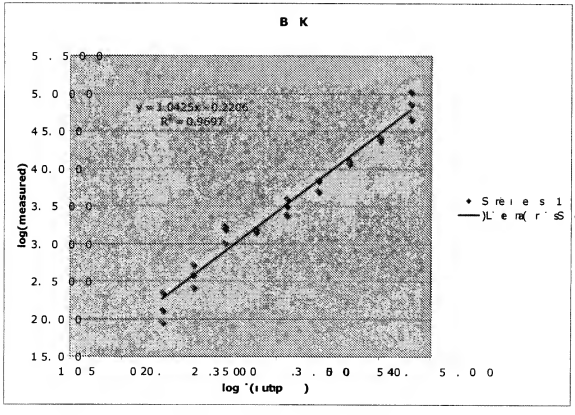


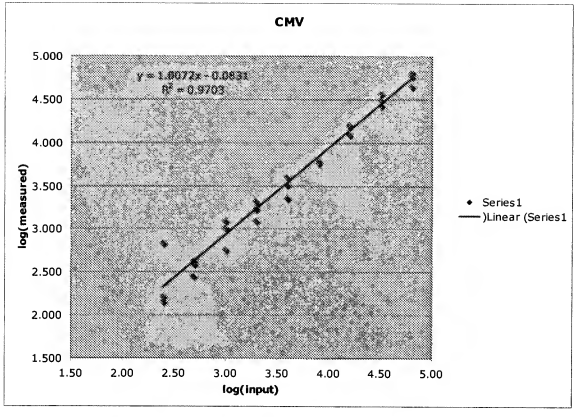
Table 2: Measured Threshold cycles for linearity validation
Triplicate sets of experiment are shown. The measured Cts were sorted for the same input concentration.

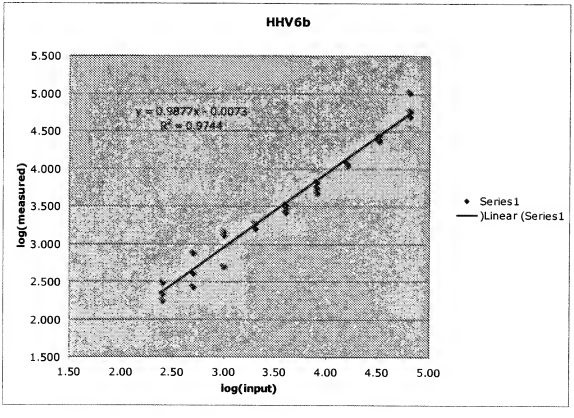
		Measured Cts			
input copy #	log(input)	BK	HHV7	CMV	HHV6b
62500.0	4.80	21.049	22.878	23.633	21.935
31250.0	4.49	21.955	23.689	24.425	22.962
15625.0	4.19	22.893	24.478	25.592	24.22
7812.5	3.89	24.243	25.917	26.951	24.952
3906.3	3.59	25.282	27.207	28.24	25.945
1953.1	3.29	26.005	27.424	28.385	26.945
976.6	2.99	26.503	28.022	30.155	27.229
488.3	2.69	28.476	29.989	31.112	29.411
244.1	2.39	29.963	30.641	32.05	29.72
62500.0	4.80	19.831	22.176	23.77	21.123
31250.0	4.49	21.971	23.677	24.631	23.198
15625.0	4.19	22.804	24.639	25.549	24.154

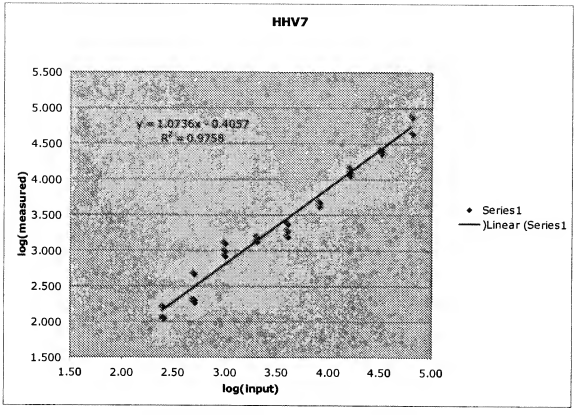
7812.5	3.89	23.74	25.799	26.902	25.195
3906.3	3.59	24.579	26.664	27.459	26.244
1953.1	3.29	25.999	27.442	29.104	26.714
976.6	2.99	25.882	27.812	29.104	27.077
488.3	2.69	27.438	28.801	30.566	27.995
244.1	2.39	29.451	30.69	29.915	30.01
62500.0	4.80	20.377	22.17	24.142	22.137
31250.0	4.49	21.833	23.577	24.841	23.097
15625.0	4.19	22.988	24.341	25.905	24.162
7812.5	3.89	23.703	25.745	26.901	25.407
3906.3	3.59	24.851	26.956	27.764	26.085
1953.1	3.29	25.907	27.265	28.65	26.923
976.6	2.99	25.745	27.482	29.333	28.548
488.3	2.69	27.903	29.887	30.682	28.86
244.1	2.39	28.674	30.197	31.858	29.235

Figure 2: Linearity of measured viral load in multiplex assay. Viral loads calculated based on the calibration plots were plotted versus estimated input viral load.




CMV
y = 1.0072x - 0.0631
R² = 0.9703
log(measured)
log(input)
5.000
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
Series1
Linear (Series1)


HHV6b
y = 0.9877x - 0.0073
R² = 0.9744
log(measured)
log(input)
5.500
5.000
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
Series1
Linear (Series1)


HHV7
y = 1.0736x - 0.4057
R² = 0.9758
log(measured)
log(input)
5.500
5.000
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
Series1
)Linear (Series1